

EXHIBIT 18

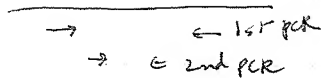
mead
COMPOSITION

Kate Kim

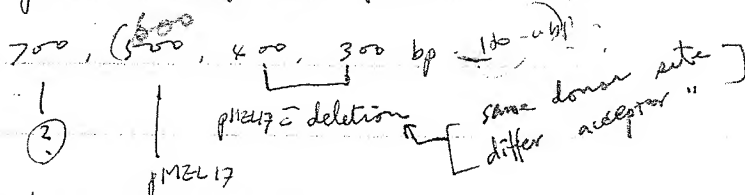
100 sheets • 200 pages
9¾ x 7½ in/24.7 x 19.0 cm
wide ruled • 09910

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pmel 17



PCR = chicken gene homologous to pmel 17 : Japan
human melanocyte RNA

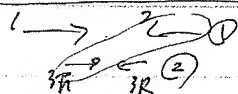


run gel [700]

get 700 / 1 → 900bp

look
P26

4-1BB



get Jurkat 500 → (filter already made high stringent
Southern [human, Gibbon, mouse DNA]
Genomic DNA cut = R1

500 - cloned partially seq.

380 380 → cloned but (?) pHA-stimulated human PBL T cell

300 300 Ribosomal binding protein

200 → (?)

Jurkat

Gibbon

① MHA poly A⁺ (Gibbon T cell)

② Jurkat (human T)

③ Molt 4 (human T)

10-135

MLA poly A + { 1 + 2
" " { 1 + 3R
" " { 2 + 3R

" Total RNA { 1 + 2
" " { 1 + 3R
" " { 2 + 3R

Molt 4 {
" "
" "

R8 ~~poly A~~ Total RNA 1 + 2

Negative control

10 μ l each, 100 ~ 400 bp

15 x 20 cm gel (Bio-Rad) in TBE, 150 3x4 hr
100

{ 1% Agarose
1.5% SeaPlaque

run until front dye is out

start 12:20 at 104 V 50 mA

12:45 106 V 56 mA

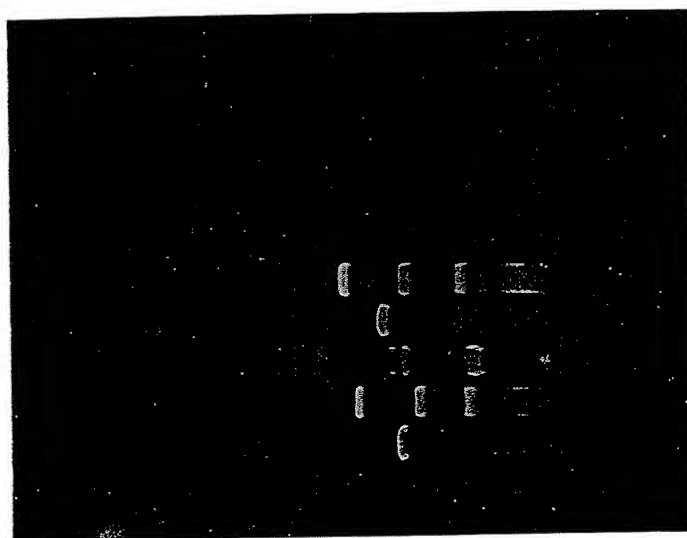
5

18:20 staining (for 30 min)

18:40 denaturation

19:30

KWON000132



unint CDMS
BstXI ant CDMS
λ marker
unint PXM
R2 cut pxm

KWON000133

Vector preparation

pxM ~~by 5000~~ cut = EcoRI

plasmid 20 μ l (20 ng)

REnt 3 10 μ l

EcoRI 5 μ l (50 units)

water 65 μ l
100 μ l

10:45 ~

COM 8 cut = BstXI

plasmid 20 μ l

NEB buffer 3 10 μ l

water 65 μ l

BstXI 5 μ l
100 μ l

11:28 ~

at 55°C

12:20

CIP treat $\frac{1}{4}$

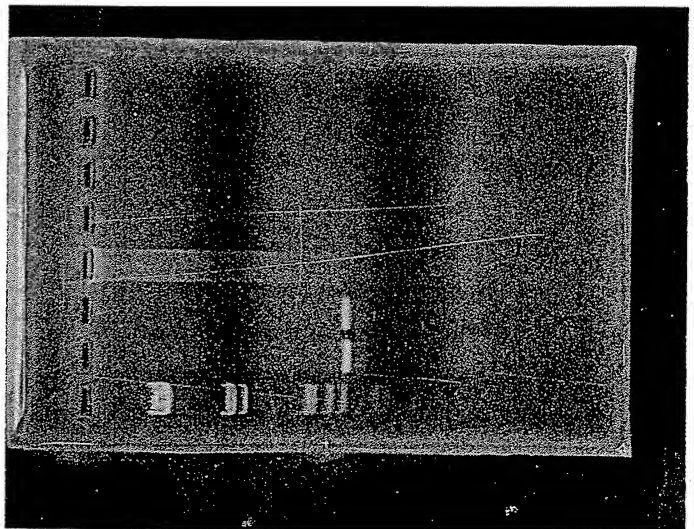
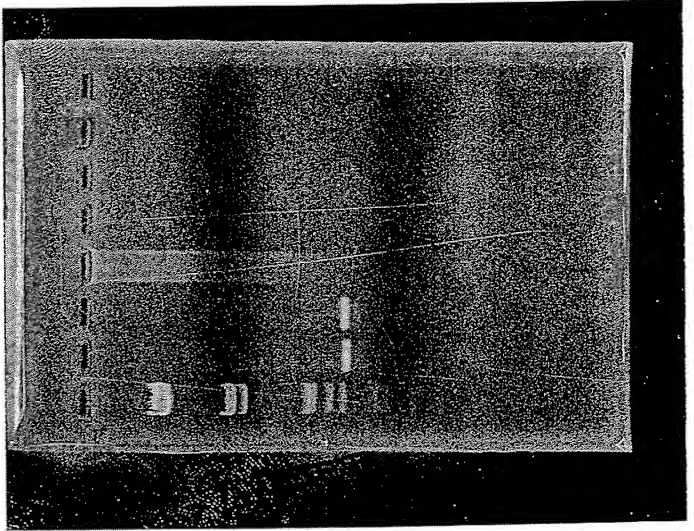
- 68°C 45 min in the presence of 10 mM EGTA

- hot phenol 60°C extraction 5 min twice

- chloroform extraction at R.T.

- Goh prep.

1. Negative control
- 2 silver - New 180ul
- 3 " " 30ul
- 4 " old 30ul
- 5 heterozygote
- 6 C57BL
- 7 C3H
- 8 X mouse 5ml (10mg)



KWON000136

(if concentration is 1 mg/ml) then $\frac{1}{\#b \times \frac{660}{2}} \times 10^6 = \text{KM} = \text{pmole/ml}$ 9

$\left(\frac{3081.7}{\#b} \right)$

PCR

Y02028 buffer 10X

Y02016 MgCl₂ 50mM

Silver-old

Silver-new

C57BL

(Silver + C57BL) Fi

C3H

* 30 ml reaction each x (5 reaction + 1 negative)
= 180 ml (-6 = 174 ml)

10X buffer 18.0 ml

MgCl₂ (50mM) 5.4 ml (1.5mM final)

dNTP (2mM) 18.0 ml (0.2mM final)

primer (S1283) 1.0 ml (0.71 pmole/ml final)

" (S1284) 1.0 ml (0.69 pmole/ml final)

43.4 ml

water 129.6

Taq polymerase 1.0 ml (5 units)

174.0

divide 29 ml x 6

1. Blank 2. Silver-new 3. Silver-old 4. C57BL 5. Fi 6. C3H
genomic DNA 1 ml

KWON000137

Dr. Park's # 8, 10, 26 + two more

50ng/ml final conc
= 5 samples

silver = 50ul + 350ul of TB/spi/protase K buffer

→ 65°C > 1 hr. → Chloroform extraction
→ 20% ZOH (lagging) → spooling 3 times

2 samples

hetero =

C57BL

1

C3H

1

9 samples

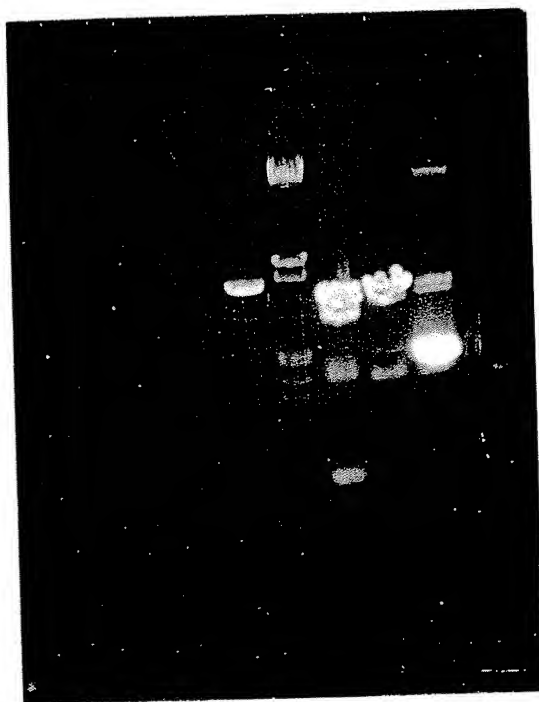
+ 1 negative control

10 samples

• protease K digestion 17:05 ~ 18:05 ~ 20:25

- ⑤ uncut Jurkat 500
- ④ Jurkat 500 cut Σ R1
- ③ Jurkat 500 cut Σ R1 & HII
- ② λ marker 250 ng (5 μ l)
- ① CDM8/BstXI cut, purified on 5-20% KOAc (1 μ l out of 200 μ l)

① ② ③ ④ ⑤



300 ng / μ l \times 200

- 6 μ g
60 μ l

KWON000139

Test cut pGEM 7Z+ + Juvex 500 (inactivated, in SmaI site)

2 ~~HindIII~~ and EcoRI

plasmid 30 μ l (40 ng)

React 3 10 μ l

water 55 μ l

EcoRI 5 μ l

100 μ l at 37°C 1 hr (11:25 - 12:43)

verify cut on Agarose GE.

Clontar Restriction 100 μ l

React 1 10 μ l (with React 3 \rightarrow becomes React 2)

water 85 μ l

HindIII 5 μ l

200 μ l (12:55 ~ 2:35)

- Load whole Rx mixture onto 1% Agarose

↓
cut out band

↓
load band onto 3.5% PAGE

↓
purify \rightarrow Nick translation

1506g ladder

Molt 4 total 2375

Molt 4 total 1138

Molt 4 total 112

Molt 4 total 2375

Molt 4 total 1138

Molt 4 total 112

Molt 4 total 2375

Molt 4 total 1138

Molt 4 total 112

Negative C

R8

KWON000141

labelling of 4-1BB (1.2kb) by Nucle-Translation

| | | | |
|---------------|--------------------|---------------|-----------|
| 4-1BB (1.2kb) | 1 μ l (100 ng) | 1 | 1 |
| NT buffer | 5 μ l | 5 | 5 |
| 0.1 M DTT | 2 μ l | 2 | 2 |
| 2 GTP (10 mM) | 1 μ l | 1 | 1 |
| d TTP (10 mM) | 1 μ l | 1 | 1 |
| $[^3P]$ d ATP | 10 μ l | 10 | 10 |
| $[^3P]$ d GTP | 10 μ l | - | 20 |
| DNase/pol | 2 μ l | 2 | 2 |
| Water | 18 μ l | 27 | 4:12 ~ |
| | 50 μ l | at 16°C | 1.5 ~ 2hr |
| | | 12:42 ~ 14:20 | |

$$\frac{3 \times 10^6 \text{ cpm} / \mu\text{l} \times 100 \mu\text{l} \times \frac{1000 \text{ ng}}{100 \text{ ng} \cdot \mu\text{g}}}{\cancel{\text{many many}}}$$

$$= 3 \times 10^8 \text{ cpm} / \mu\text{g}$$

hybridization 15x20 cm NYTRAN

5M NaCl 10 ml

10% SDS 5 ml

150 μ g/ml S.S. DNA (10 mg/ml \times 750 μ l) 750 μ l
 \times 50 ml = 7.5 mg

Probe 3×10^6 cpm/ μ l ~~50 ml~~ $50 \text{ ml} \times 10^6 \text{ cpm}/\text{ml}$
 $= 5 \times 10^7 \text{ cpm}$

$$\frac{5 \times 10^7 \text{ cpm}}{3 \times 10^6 \text{ cpm}/\mu\text{l}} \approx \underline{20 \mu\text{l}}$$

at 65°C O/N

Wash 1. 2XSSC + 1% SDS at R.T.

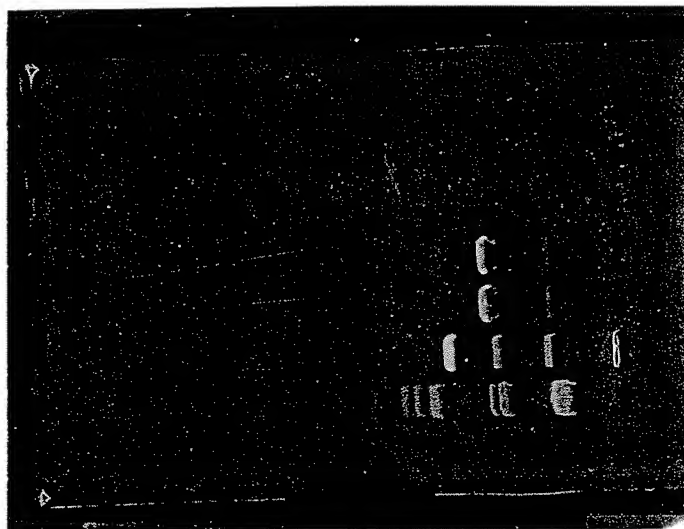
(total 500 ml)

2. 2XSSC + 1% SDS at 42°C

for 15 min.

expose film at -70°C

develop after 18 hrs



Agarose 1%

Bst XI cut (300 ng)

EcoRI cut (")

untreated pDNA 1

λ 250 ng

KWON000144

PCDNA test cut

• dilute DNA (4 ng/μl) 1 μl in TE 19 μl (1:20 dilution)

Rx 1. diluted DNA (200 ng/μl) 3 μl (600 ng)

NEB buffer 2 μl

water 14 μl

Bst XI 1 μl

20 μl

50°C 17:55

Rx 2

diluted DNA

3 μl (600 ng)

~ 20:00

REact 3

2 μl

water

14 μl

Eco RI

1 μl

20 μl

37°C 17:50

~ 20:00

Membrane strip

[0.2% SDS

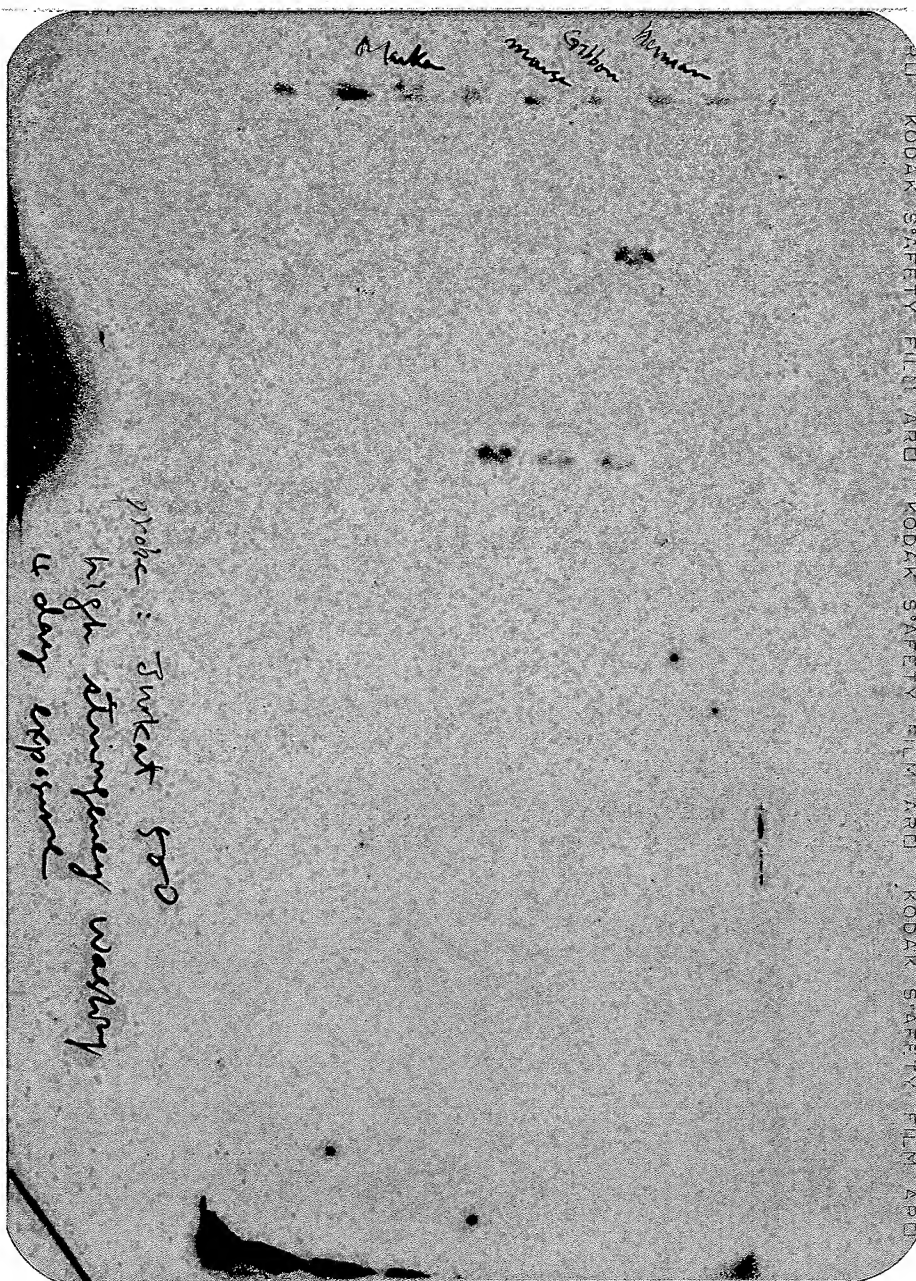
10 mM Tris pH 8.0

(50 mM by fault)

85°C 2h

20:40

~ 22:40



KWON000146

Surket
500

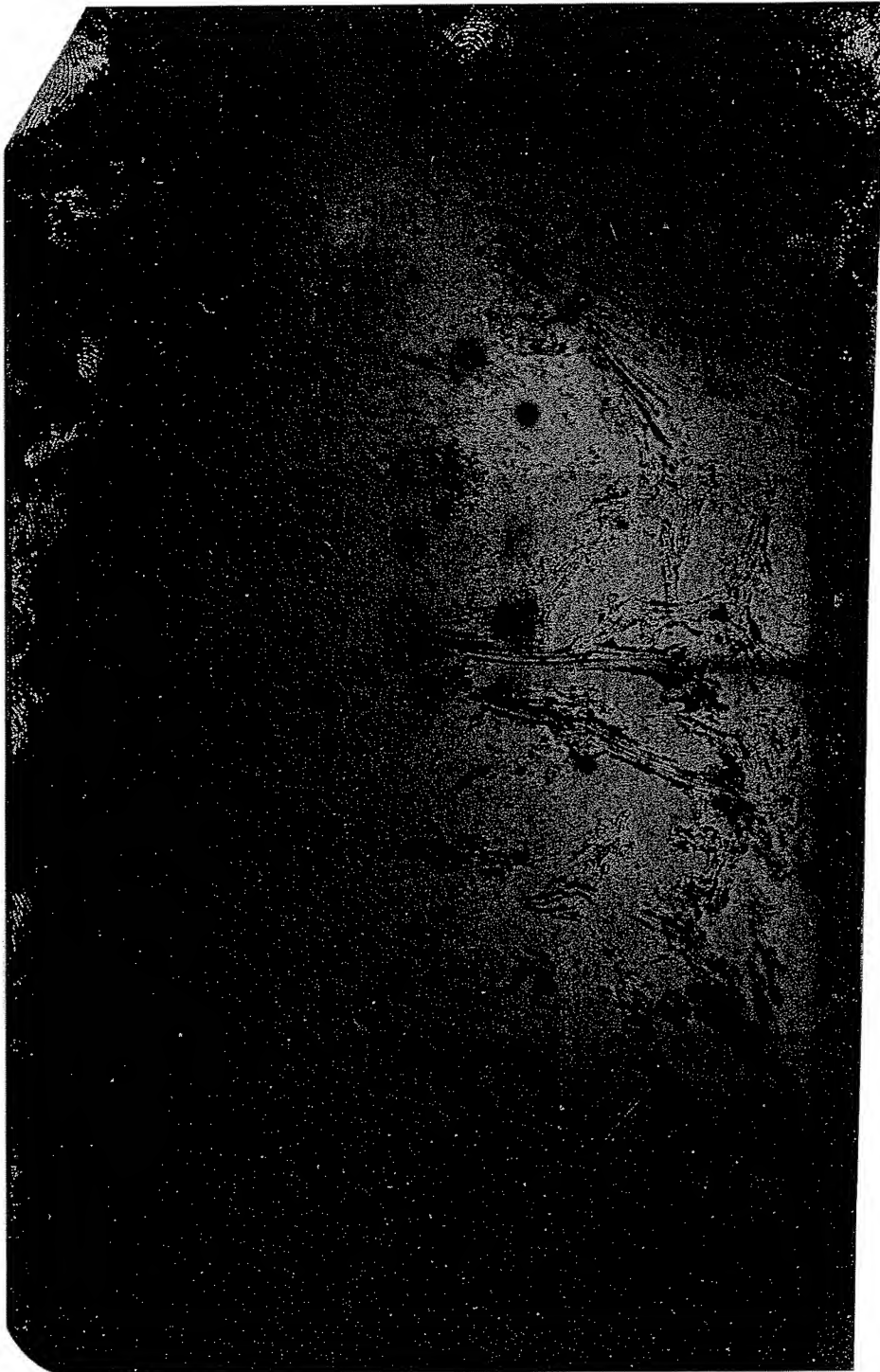
M *mokse* *G.P. Hoon* *Helen*

moÿse

GPB

Hillman

KODAK SAFETY FILM AND KODAK SAFETY FILM




KWON000148



Nick translation of Tmkat 500 pcr fragment (PAGE purified)

DNA 1 μ l (100 ng)

follows  4-IBB labelling protocol (page 15)

at 16°C 16:25 ~ 18:25

85
85

39 2003346
200 2040708

1801276

71/27/16 1/17/04/16

$$4.7 \times 10^6 \text{ cpm}/\mu\text{l} \times 30 \mu\text{l} \approx \frac{1.2 \times 10^8 \text{ cpm}}{\text{total}}$$

$$\frac{1.2 \times 10^6 \text{ cpm}/\mu\text{l} \times 25 \mu\text{l}}{4.7 \times 10^6 \text{ cpm}/\mu\text{l}} = \underline{\underline{5 \mu\text{l}}}$$

sp. act.
 $1.2 \times 10^8 \text{ cpm}/\mu\text{g}$

8X 17.5 cm membran : 140 cm² → 28 ml

$$50 \text{ ml} \times \frac{6\%}{20\%} = 15 \text{ ml (of 20X SSC)}$$

$$50 \text{ ml} \times \frac{0.5\%}{10\%} = 2.5 \text{ ml (of 10\% SDS)}$$

$$\frac{100 \mu\text{g}/\text{ml}}{10 \text{ mg}/\text{ml}} \times 50 \text{ ml} = 500 \mu\text{l (of 10 mg/ml S.S.-DNA)}$$

cycle profile

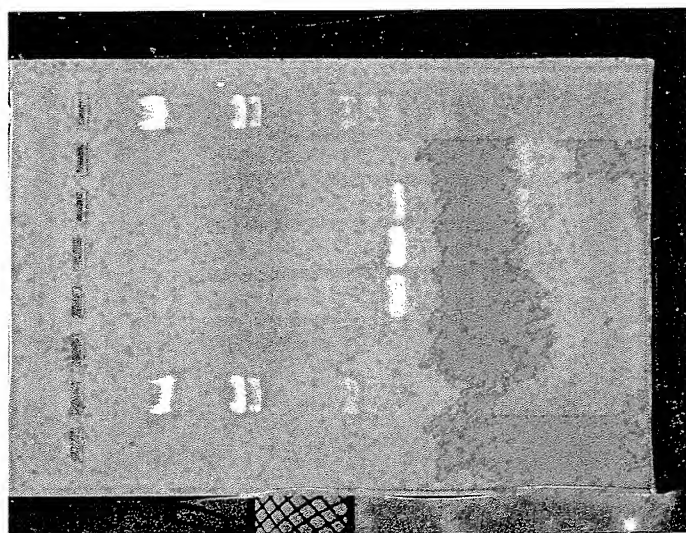
14. 94°C 2min

15. 94°C 1min 55°C 1min 72 1min

16. 94°C " " " " 2min

17 72°C 10min

7 25°C



KWON000150

PCR

template ① Silver

(9) ② hetero

silver (Dr. Park's # 1, 8, 11, 26, 33)

③ C57BL

④ C3H

$$30 \mu\text{l}/\text{reaction} \times (9 \text{ reactions} + 1 \text{ negative control})$$

$$= 300 \mu\text{l} (- 1 \mu\text{l} \text{ template} \times 10 \text{ template} = 290 \mu\text{l})$$

Master mix

10X buffer 30.0 μl

MgCl₂ (50mM) 9.0 μl (1.5mM final)

dNTP (10mM) ~~12.0~~ 6.0 μl (0.2mM ")

primer (S1283) 2.0 μl (~0.9 pmole/ μl)

" (S1284) 2.0 μl (")

Subtotal 49 ~~55~~ μl

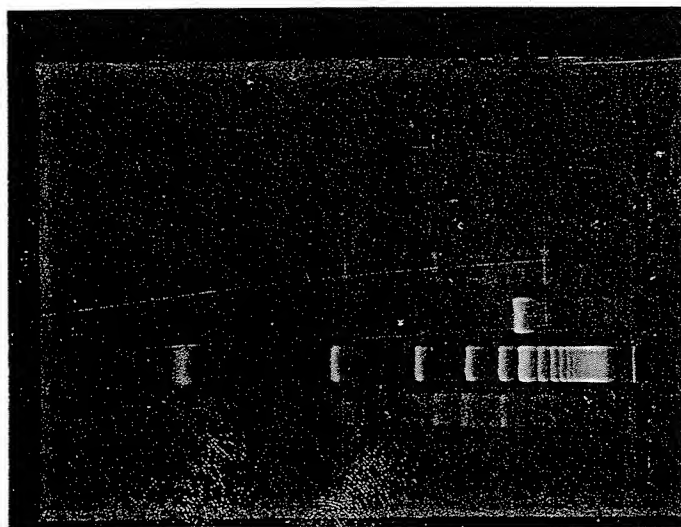
Tag 2.0 μl (10 units)

water ~~23.0~~ 23.0 μl

290.0 μl

- divide 29 μl into 10 tubes that contain 1 μl template on the wall
- add paraffin oil (3 drops)
- vortex \rightarrow spin \rightarrow cycle

MIP Brent
Brent steel



Brent 200

steel @ 1166 ✓

④ 480 ✓

② 380

MIP @ 900

② 750

② 700

② 550

330

310

220

200

150

A

PAGE purification of Steel, Brent (pmel17), and MIP PCR

EtOH ppt of 100 μ l of PCR Rx. \rightarrow redissolve in 20 μ l water.
 (add Glycogen or linear PA)

| | | | |
|---------|---------|---------|---------|
| ┌┐ | ┌┐ | ┌┐ | ┌┐ |
| 1.5 | 1.5 | 1.5 | 1 |
| μ m | μ m | μ m | μ m |

Steel Brent MIP (adder)

polishing the end (as in [redacted])

DNA 20 μ l (in b.p.w.)10X buffer 10 μ lwater 68 μ lKinase 1 μ lKlenow 1 μ l

master mix

$$80 \mu\text{l} \times 13 = 1040 \mu\text{l}$$

$$100 \mu\text{l} (\times 13 = 1300 \mu\text{l})$$

10X buffer 130 μ l

water 890

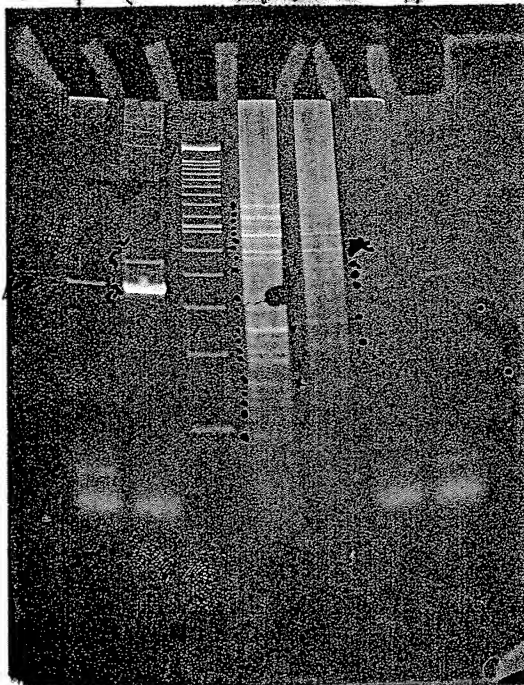
Kinase 10 μ lKlenow 10 μ l1040 μ l

(6)

8:45 ~ 9:45

mouse photo 17-10-85

1 5 01 Top 2 4



1 1 - <100

1. <100, 1-26

KWON000154

PCR

template

- ① silver ② hetero ③ c57BL ④ silver cDNA ⑤ ^{mouse} pMZL17 cDNA

$$100 \text{ nl/reaction} \times (5 \text{ reactions} + \text{negative control}) \quad (\text{half vol.})$$

$$= \frac{500}{500} \text{ nl} \quad (-1 \text{ nl} \times 5.0 \neq 5.0 \text{ nl})$$

master mix

| | |
|--------------------------|------------------------------|
| 10x buffer | 50 55 nl |
| MgCl ₂ (50mM) | 20 22 nl (2mM final) |
| dNTP (10mM) | 10 11 nl (0.2mM →) |
| primer (S1283) | 4 nl (~0.9 pmole/nl) |
| primer (S1284) | 4 nl (") |
| subtotal | 88 96 nl |
| Tag | 3 nl (10 units) |
| water | 404 446.5 495 546.5 |

divide 99 nl each (x5) ~~not 50 nl~~

5:03 ~ 5:35 ~ 6:18

Preparations for cDNA synthesis

1. PXM/RI CIP treatment

~ 20 mg PXM/RZ (page 5) P/E extracted & EOH ppt
 dissolved in 90 μ l of Tris (pH 8.4) ^{according to Maniatis}
 aliquot 1 μ l and save (pH 8.3)

add 10 μ l CIP buffer (10 mM ZnCl₂
 10X (10 mM MgCl₂
 100 mM Tris (pH 8.4))

add 1 μ l (1 unit/ μ l) of BM CIP
 incubate at 37°C for 30 min.

add 2 μ l of 0.5M EGTA (final 10mM)
 and incubate at 68°C for 45 min (or 65°C for 1 hr)

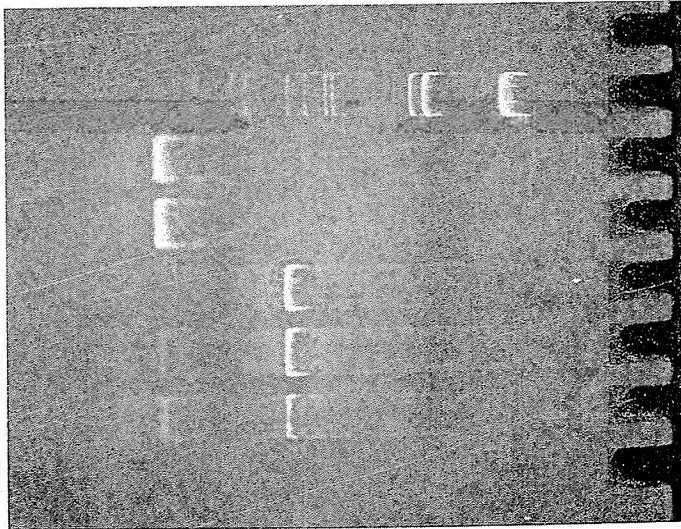
add pre-heated (55°C) phenol/chloroform,
 vortex and incubate at 55°C for 5 min.

spin and transfer upper aq. layer to new tube

→ repeat

EOH ppt

5 4 3 2 1



KWON000157

PCR repeat (page 25)

template ① silver ② hetero ③ C57BL ④ silver DNA ⑤

⑤ mouse pM2C17C DNA

Reaction

volume ~~add~~ same as page 25

Cycle profile

1 cycle user 14 94°C 2 min

4 cycle user 15 94°C 1 min 50°C 1.5 min 72°C 2 min

11 cycle user 16 94°C 1 min 53°C 1.0 min 72°C 1 min

15 cycle user 17 94°C 1 min 55°C 1.0 min 72°C 2 min

1 cycle user 5 72°C 10 min

1 cycle user 7 25°C R.T.

50 μ l/reaction \times 5 reactions

= 250 μ l (- 1 μ l/template \times 5 templates = 245 μ l)

master mix 10X buffer 25 μ l

MgCl₂ (50 mM) 7.5 μ l (1.5 mM final)

dNTP (10 mM) 5 μ l (0.2 mM each)

primer (S1283) 2.0 μ l (1 pmole/ μ l)

" (S1284) 2.0 μ l (")

subtotal 41.5 μ l

Tag 2.0 μ l

water 201.5 μ l

245.0 μ l

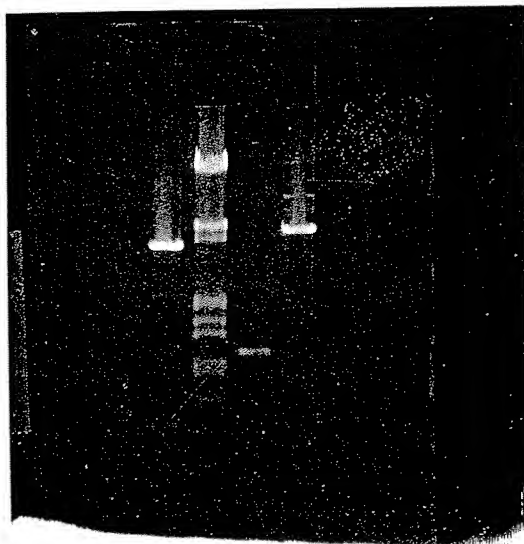
(divide 49 μ l \times 5 tubes

add 1 μ l of template

add paraffin oil

| SAMPLE | A320 | P280 | A2 |
|--------|--------|---------|-----|
| 1.0000 | 0.0000 | -0.0000 | 0.0 |
| 2.0000 | 0.0043 | 0.0777 | 0.1 |
| 3.0000 | -0.001 | -0.001 | -0. |
| 4.0000 | -0.001 | 0.0477 | 0.0 |
| 5.0000 | -0.001 | 0.0480 | 0.0 |
| 6.0000 | 0.0046 | 0.0034 | 0.0 |
| 7.0000 | 0.0040 | 0.0030 | 0.0 |
| 8.0000 | 0.0077 | 0.1348 | 0.2 |
| 9.0000 | 0.0075 | 0.1344 | 0.2 |

CDM 8
4-1BB/R1
PXM



CDM 8: Stuffer remain?
4-1BB/R1
PXM: some unmet
remains

KWON000159

Test ligation of CIP T α PXM/RI vectors
 CDM8/B β TXI

| | | |
|-----------------------------|--------------|--------------|
| 1. PXM/RI (111 ng/ μ l) | 1.0 μ l | 1.0 μ l |
| 4-IBB (15.7 ng/ μ l) | 1.7 μ l | — |
| 5X BRL buffer | 4.0 μ l | 4.0 μ l |
| T4 DNA ligase | 1.0 μ l | 1.0 μ l |
| water | 12.3 | 14.0 μ l |
| | 20.0 μ l | 20.0 μ l |

Vectors are not prepared well!!

↓

Repurified \rightarrow p39

Dot blot of MLA ^[Total RNA] poly A PCR products

→

| | | | | | | | | | | | | | |
|-------|-----|------------------|--------|--------|-----|--------|-----|-----|-----|-----|-----|--|-------|
| 4-1BB | PXM | pcDNA | pcDNA8 | Ladder | λ | poly A | → | | | | | | |
| 210 | 220 | 240 | 295 | 320 | 410 | 490 | 490 | 530 | 570 | 600 | 650 | | |
| 700 | 780 | 220 | 270 | 350 | 380 | 410 | | | | | | | 4-1BB |

3ul each of PCR product (out of 20ul) dotted

4-1BB 50 ng

PXM 100 ng

pcDNA8 200 ng

Ladder 300 ng (0.3ul)

λ 150 ng

after application, float on D.B.W

2. denature for 5'

3. neutralization for 5'

4. rinse in 2XSSC

5. partially dried → Statalink

| SAMPLE | A320 | A280 | A260 | 280/240 | 260/280 | PROTEIN | NUCLEIC ACID |
|--------|------|------|------|---------|---------|---------|--------------|
|--------|------|------|------|---------|---------|---------|--------------|

| | | | | | | | |
|--------|--------|--------|--------|--------|--------|--------|--------------|
| 1.0000 | 0.0000 | 0.0000 | 0.0000 | ***** | ***** | 0.0000 | 0.0000 |
| 2.0000 | 0.0188 | 0.0379 | 0.0462 | 0.4464 | 2.2401 | 2.2197 | 22.197 mg/ml |
| 3.0000 | 0.0000 | 0.0000 | 0.0000 | ***** | ***** | 0.0000 | 0.0000 |
| 4.0000 | 0.0050 | 0.1120 | 0.2143 | 0.5062 | 1.9753 | 2.4350 | 97.536 mg/ml |
| 5.0000 | 0.0021 | 0.0023 | 0.0026 | 0.9462 | 1.1010 | 0.0169 | |
| 6.0000 | 0.0023 | 0.0026 | 0.0036 | -0.182 | -5.500 | -0.515 | 0.0611 |
| 7.0000 | 0.0013 | 0.0000 | 0.0000 | 1.0000 | 1.0000 | -1.017 | -0.034 |
| 0000 | 0.0051 | 0.0432 | 0.0812 | 0.5005 | 1.9979 | 3.4146 | 34.146 " |
| 00 | 0.0000 | 0.0000 | 0.0000 | ***** | ***** | 0.0000 | 0.0000 |
| 0 | 0.0141 | 0.1060 | 0.1913 | 0.5128 | 1.9501 | 7.7858 | 27.858 " |

0.0000 DNA: 0.00

2.2197 6:57

0.0000 6:64

2.4350 6:57

0.0169 6:57

-0.515 6:57

-1.017 6:57

3.4146 6:57

0.0000 6:57

7.7858 6:57

KWON000162

ligation of BstXI cut pCDM8 & pCDNA1
with adapted pVUT fragment of pMEL17 (or pXM5)

1. Adaptor ligation

pVUT fragment (22 ng/ul) 2 ul

BstXI adapter (0.5 ng/ul) 1 ul

5X BRL ligation buffer 4 ul

water 12 ul

T4 ligase 1 ul

20 ul at 16° 1 hr

11:42 ~ 01:00

• at 65°C 10 min

• add NaI (gene clean kit) 150 ul

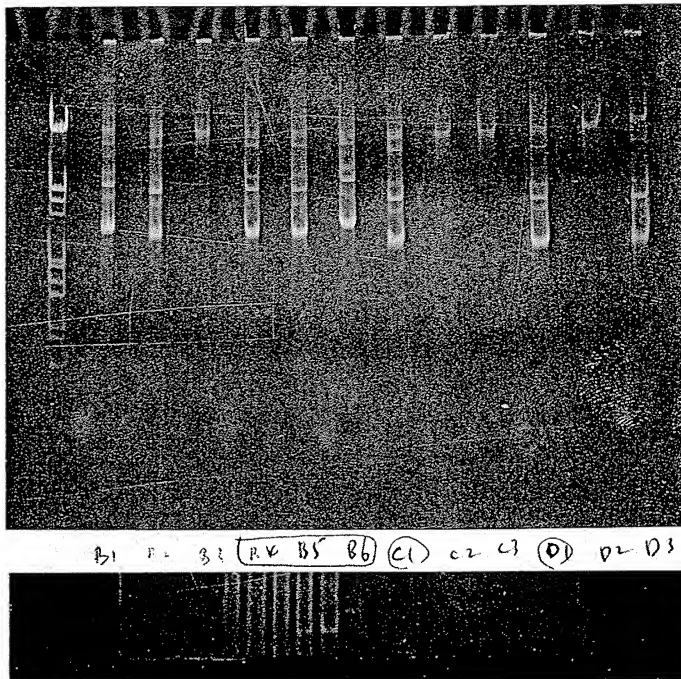
• add 2 ul of glassmilk (0.1:17)

• follow gene clean procedure

• elute twice → total 20 ul

CDNA

transfo
Invitro
add
divi
on
add
on



0.3ml
(0.3ml)
μl
tubes
cleaned)

heat shock at 42°C water bath for 65 seconds
on ice for > 2 min.

add $\frac{300}{250}$ μl of SOC medium (provided by Invitrogen)

37°C on the wheel for 1 hr.

plate whole thing on Amp-LB plate

(g. h; before plating add 3ml LB)

and plate 100 μl each

100ng before

25ng

40

104000

1x10⁵/μg

a: nothing ~~PCDNA8~~ 261

b: ~2600

c: 335

d: 279

f: 205

g: ~560 x 32.5 = 18,200/ng

h: nothing

a: eg cDNA
b: h: PCDNA

10⁷/μg

KWON000164

Ligation of adaptor-pme17/pvuII = { CDM8
pCDNA1

1. gene-cleaned adaptor-pme17/pvuII = 10 μ l (~20 ng)

① CDM8 (97 ng/ μ l) ② pCDNA1 (34 ng/ μ l) 1 μ l 3 μ l

5X ligation buffer (BRL) 4 μ l 4 μ l

water

ligase (T4 DNA ligase, BRL) ③ $\frac{1 \mu\text{l}}{20 \mu\text{l}}$ $\frac{1 \mu\text{l}}{20 \mu\text{l}}$

vector alone 4 μ l 2 μ l
+ self-ligation (14) (12)

at 16°C

* control: pme17/pvuII in place of adaptor-pme17/pvuII

pme17/pvuII (22 ng/ μ l) 1 μ l 1 μ l

① CDM8 (97 ng/ μ l) ② pCDNA1 1 μ l 3 μ l

5X ligation buffer 4 μ l 4 μ l

water 1 μ l 11 μ l

ligase $\frac{1 \mu\text{l}}{20 \mu\text{l}}$ $\frac{1 \mu\text{l}}{20 \mu\text{l}}$
at 16°C

2. Transform

[CDM8
pCDNA1] X { vector alone
vector + frag.
vector + adaptor + frag.
uncut vector (1 ng)

④ CDM8

⑤ pCDNA1

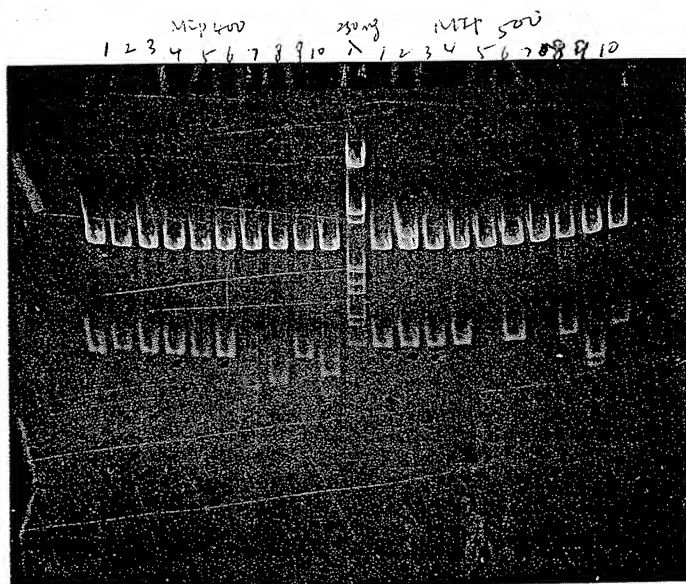
80%

KWON000165

ligation of pXM/R1 · CIP

| | | | |
|--------------------------|-----------------------------|-------------|---------------------------|
| 1. pXM/R1 (78 ng/ul) CIP | ^(155 ng) 2 ul | 2 ul | ^{130 ng} 1 ul |
| 4-1 BB (16 ng/ul) | 2.5 ul | - | - |
| 5X BRL ligation buffer | 4 ul | 4 | 4 |
| water | 10.5 | 13 ul | 14 |
| T4 ligase (BRL) | 1 ul | 1 | 1 |
| | <hr/> 20.0 ul | <hr/> 20 ul | <hr/> 20 ul |

⊛ pXM/R1 CIP - not Tx 1 ul



MCP 500 : 1, 2, 3, 4, 6, 8, 10 MCP 400 : 1

: 9

: 2, 3, 4, 5, 6, 9

: 5, 7 (no insert)

: 7

: 8

: 10

KWON000167

digestion of MIP 400 & MIP 500 clones (10 each ~~x~~ 2)

• Mastermix I for $20 \mu\text{l} \times 20 = 400$ (- 5 μl of miniprep $\times 20$)

React 3 40 μl

water 240 μl

EcoRI 20 μl

300 μl

• divide into 20 used & washed tubes

• add 5 μl of minipreps

• mix and at 37°C for 2 hr

• take 10 μl separate

Into remaining 10 μl add 10 μl of mastermix 2
master mix 2

React 1 20 μl

water 170

HindIII 10 μl

200 μl

mix and incubate for 1 hr at 37°C

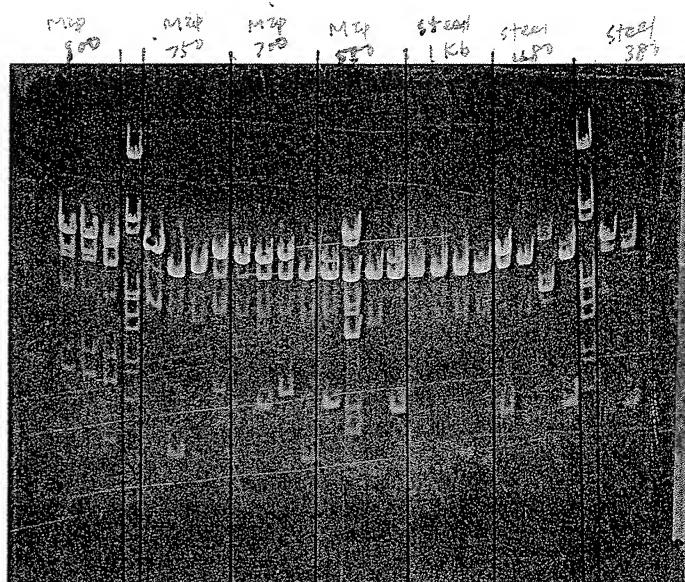
take 10 μl and run gel

* Transform XL-1 blue \pm ligation mixture of
polished PCR products of page 77 & 47

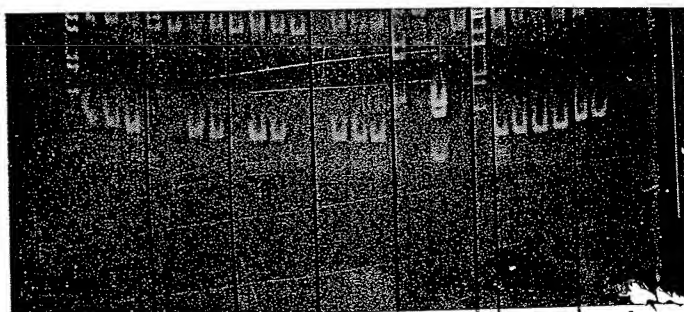
page 77 (Steel ⁱ 1Kb, ^h 480, ^g 380
MSP ^m 300, ^e 750, ^k 700, ^j 530

page 47 (\Rightarrow)

* pick 4 colonies ~~each~~ from each plate
prepare plasmid
digest with



M43/ L4-1 K44-1 J4-1 K4-1 L4-1 M4-1 N4-1



a123 b1-4 c1-4 d1-4 e1-4 f1-4 g1-2

KWON000169

ligation of pcr products
 Steel 1Kb, 480, 380 (7 fragment)
 [MZP 570, 700, 750, 900

ligation

~~7x~~ 20ul = 140 ul (- ~~10~~ 10^{ul} x 7 = 70ul)

5x buffer 28 ul

vector 1 ul (pGreen3/Sma2 CIP 6)

water 36 ul

T4 ligase 5 ul

70 ul

divide into 7 tubes (10ul each)
 add pcr frag. (10ul each)

at 20°C 65°C 1hr

ligation of pcr products from [] and []

[] Silver genomic 1-2 (350bp)
 (page 29)

mouse pMEL17 CDNA 350bp 450bp
 5-1, 5-2

[] Silver cDNA 4 (350bp)

(page 33)

silver genomic 1 (1.2Kb and 350bp)

6 x 20 ul = 120 ul (- 10 ul x 6 = 60 ul)

a 1-2 half (10ul)

b 5-1 2 ul

c 5-2 half (10ul)

d 4 2 ul

e 1 (1.2Kb) half

f 1 (350) half

5x buffer 24 ul

vector 1 ul

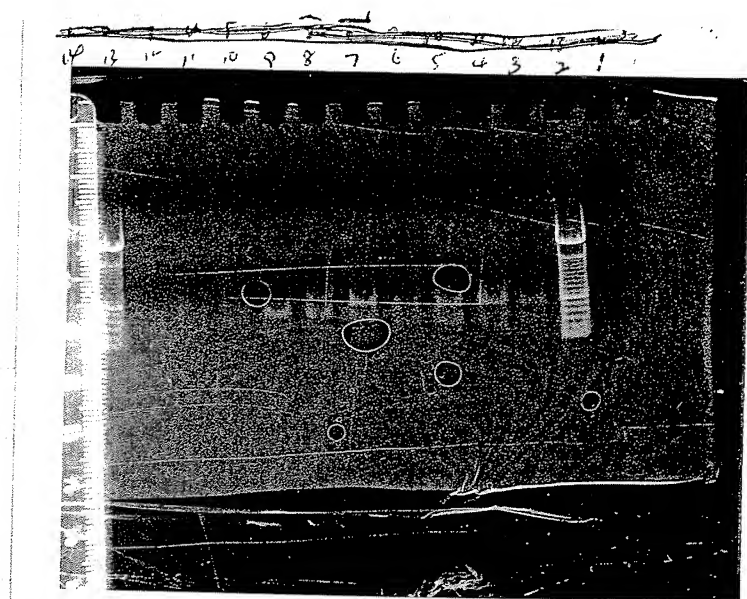
water 32 ul

T4 ligase 3 ul

60 ul

divide into 6 tubes 10 ul each
 add repaired frag.

at 20°C



prehybridization

6X SSC

5X Denhardt

1% SDS

150 μ g/ml ssDNA

at 7:20 at 65°C

8:20

hybridization

6X SSC

5X Denhardt

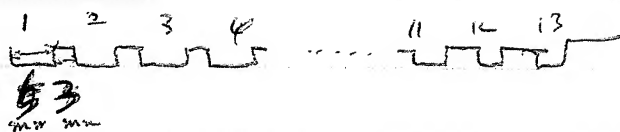
1% SDS

150 μ g/ml ssDNA

4-18B probe 5×10^6 cpm/ml

at 37°C

KWON000171



1. ~~ladder~~ 4-1BB

2. ~~4-1BB~~ ladder

3. 98 220 550 poly

4. 110 240 ~~550~~ 570 poly A⁺ min 7 ul ~~of each frag~~

5. 120 295 600

6. 135 320 650

7. 150 410 700

8. (190) 470 780

9. (210) 490

10. 220 380

11. (270) 410

12. 350

total RNA

13. ~~4-1BB~~ ladder

14. 4-1BB

| SAMPLE | A320 | A280 | A260 | 280/260 | 260/280 | PROTEIN | NUCLEIC ACID |
|--------|------|------|------|---------|---------|---------|--------------|
|--------|------|------|------|---------|---------|---------|--------------|

| | | | | | | | |
|--------|--------|--------|--------|--------|--------|--------|--------|
| 1.0000 | -0.001 | 0.0000 | 0.0010 | 0.5098 | 1.9615 | 0.0492 | 0.0909 |
| 2.0000 | 0.0049 | 0.0424 | 0.0801 | 0.4989 | 2.0043 | 1.2821 | 3.3774 |
| 3.0000 | -0.001 | -0.001 | 0.0000 | -0.080 | -12.50 | -0.881 | 0.0658 |
| 4.0000 | 0.0293 | 0.0520 | 0.0678 | 0.5894 | 1.6766 | 6.0585 | 1.6039 |
| 5.0000 | -0.001 | 0.0000 | 0.0000 | 1.0000 | 1.0000 | 0.9536 | 0.0323 |
| 6.0000 | 0.0119 | 0.0201 | 0.0300 | 0.4523 | 2.2108 | -0.997 | 0.8410 |
| 7.0000 | 0.0112 | 0.0205 | 0.0295 | 0.5098 | 1.9614 | 0.6212 | 0.8143 |
| 8.0000 | -0.002 | -0.002 | -0.002 | -2.000 | -0.500 | -0.618 | 0.0216 |
| 9.0000 | 0.0174 | 0.0338 | 0.0488 | 0.5222 | 1.9148 | 1.6751 | 1.3883 |
| 10.000 | 0.0181 | 0.0340 | 0.0495 | 0.5077 | 1.9699 | 0.9589 | 1.3994 |

pRC/CMV (BstX1) = water
 2.5 : 57.5 \Rightarrow 84 ng/ μ l

pM2117/pVU2 BstX1:
 water
 5 : 55 \Rightarrow 16.8 ng/ μ l

KWON000173

cut pRC/CMV \pm BstXI

plasmid 15 μ l (15 μ g)

NEB #3 10 μ l

water 70 μ l

BstXI 5 μ l

100 μ l

at 50°C

~~add BstXI~~

~~10 min~~
90

ligation

| | ① | ② | ③ | ④ | ⑤ | ⑥ |
|--------------------|-----------|-----------|----------|----------|--------|----------|
| pCDM8 | 1 μ l | 1 μ l | - | - | - | - |
| pRC/CMV | - | - | 1 | 1 | - | - |
| pCDNA1 | - | - | - | - | - | - |
| pRC/CMV | - | - | - | - | 2.5 | 2.5 |
| 5X ligation buffer | 4 | 4 | 4 | 4 | 4 | 4 |
| primer 17 | 1 | - | 1 | - | 1 | - |
| PvuII/BstXI | 11.5+1.5 | 11.5 | 11.5+1.5 | 11.5+1.5 | 11.5+1 | 9+1.5 |
| water | 13 | 14 | 13 | 14 | 11.5 | 12.5 (7) |
| ligase | 1 | 1 | 1 | 1 | 1 | 1 |
| | 20 | 20 | 20 | 20 | 20 | 20 |

Master mix $20 \times 6 = 120$ { $-(1+2.5) \times 6 = 18$ } $\frac{99}{102}$

5X buffer 24

water 69

ligase 6

~~102~~ 99 μ l 16.5

KWON000174

Todo

1) stilling 1 kb.

Dr. K. M. has ligate

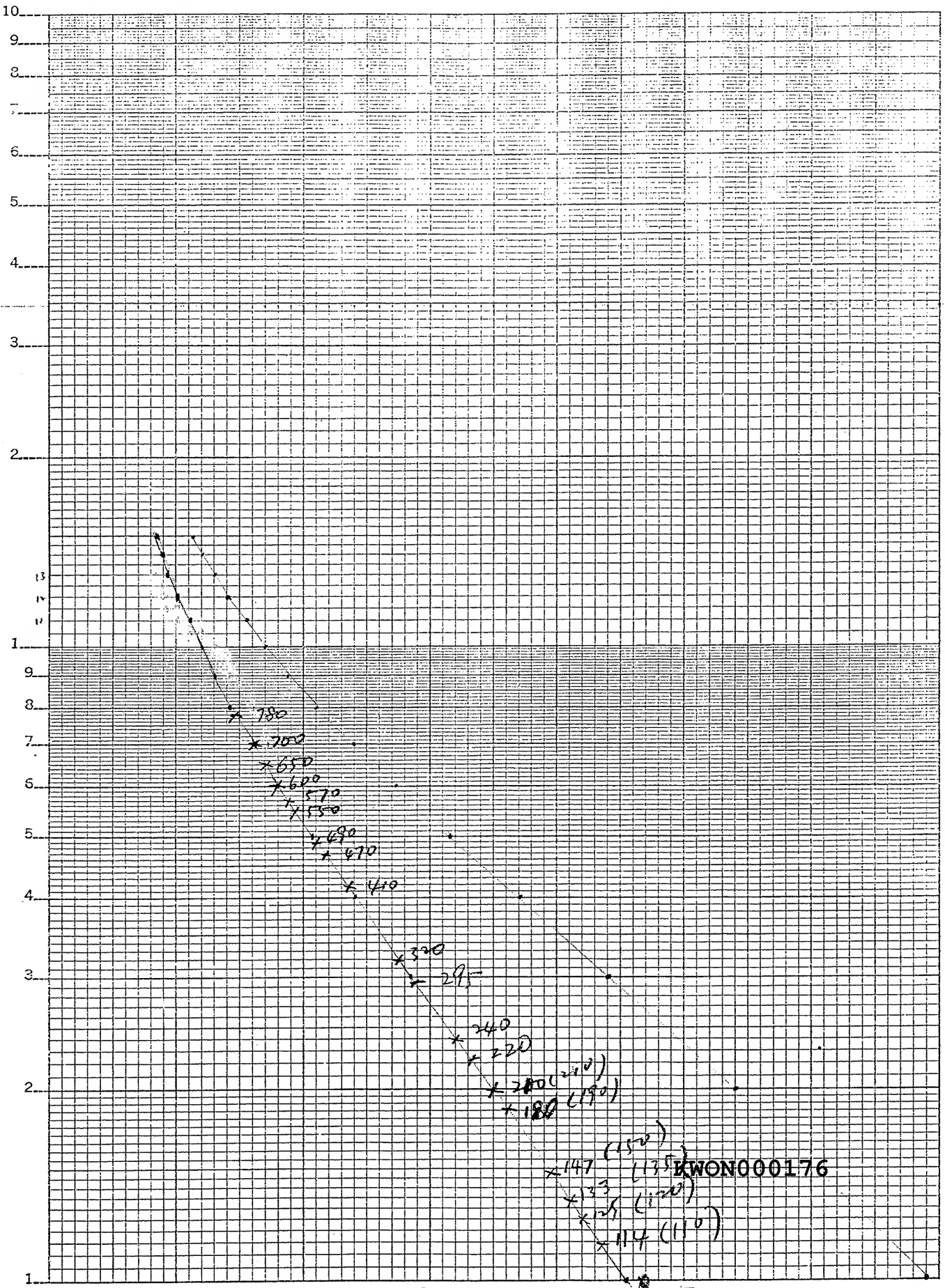
plate X2-1 blue

2) all the fragments of MIP-PCR
stilling has been repaired
& cloned

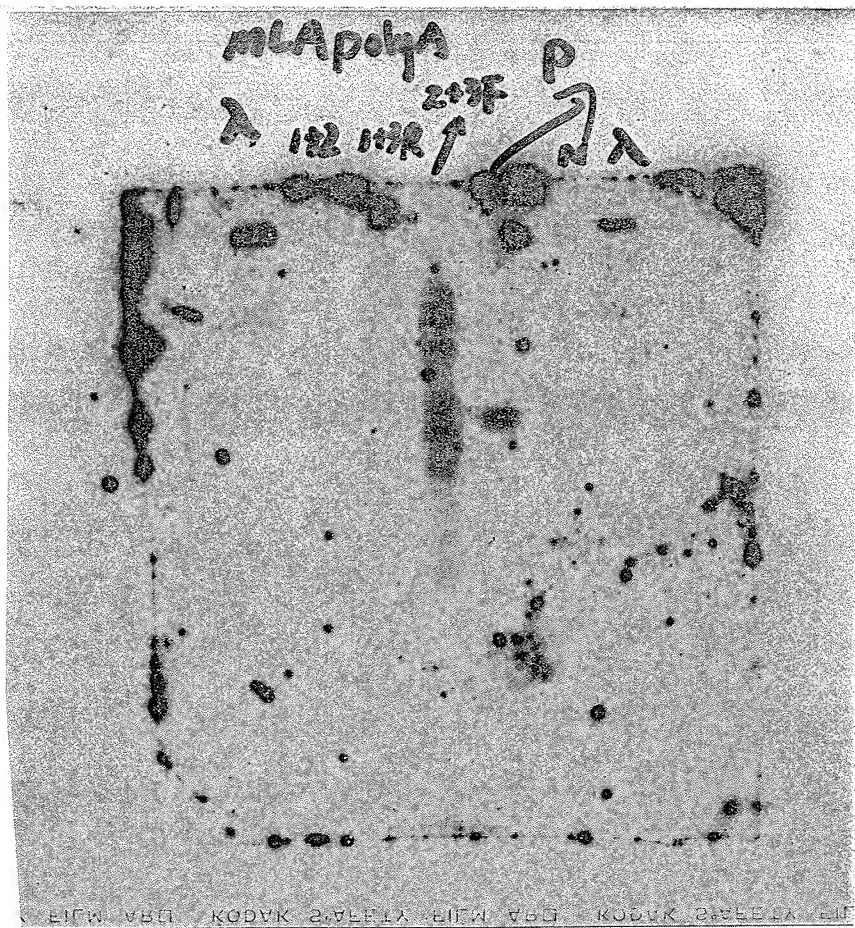
KWON000175

11

Kakkeeyunum "



KWON000176



KWON000177